

Allergenicity suppression in natural latex surgical gloves

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By a revised manufacturing process we aimed to obtain latex surgical gloves without immediate allergenicity, and also to determine a simple physical technique by which to estimate this allergenicity. Five glove samples were evaluated for allergenicity by physical and biochemical methods and by skin tests at the University Allergy Centre. Three groups of allergic patients with documented anaphylaxis to surgical gloves, positive skin test to latex extract and specific IgE to latex (RAST Pharmacia $\geq$ class II), volunteered for the study. The protein content, the *in vitro* allergenic potency of glove supernatants (RAST inhibition) and the skin test results with glove supernatants were lower in washed gloves than in non-washed samples ($P < 0.02$ to $P < 0.009$). The supplementary effect of glove sterilization at 120°C (for 1 h in saturated steam) was obvious. The protein content became undetectable in four of the five glove supernatants, and skin tests results with sample supernatants were decreased ($M \pm SEM = 0.68 \pm 0.29$ mm vs 3.06 ± 0.61 mm with non-sterilized gloves), $P < 0.009$ by Wilcoxon test. With the Spearman test there was a significant correlation ($P = 0.029$) between the mean wheal size, obtained with supernatants (Groups 1 and 2) and electrical conductivity. Skin tests through pieces of gloves randomly distributed and coded were decreased by sterilization: 0.66 ± 0.25 mm vs 2.86 ± 0.45 mm, $P < 0.0001$. Thus, it is possible to decrease glove allergenicity by washing them after mould forming and then sterilizing with steam. Electrical conductivity may be a simple parameter for revealing allergenicity.

Key words: allergenicity; anaphylaxis; electrical conductivity; latex gloves.

Accepted for publication 20 March 1991

CLINICAL ASPECTS

The frequency of immediate allergy to natural latex is high. An increased washing period during production of latex gloves was not sufficient to reduce the allergenic proteins. It was necessary to supplement with steam sterilization to obtain gloves without allergenicity as shown by RAST inhibition and skin tests in allergic patients.

Since the first two cases of anaphylaxis to latex surgical gloves were reported (12), many descriptions of others have been published (1, 2, 3, 5, 6, 7, 8, 10, 15, 17, 18). The frequency of immediate allergy to natural latex is high, particularly among people who have a medical occupation. For example, Turjanmaa et al. (13) found that 7.5% of doctors and 5.6% of nurses who worked in operating departments were allergic to latex. More recently, Tarlo et al. (11)

found positive skin tests to latex in 11% of workers in a surgical glove manufacturing plant.

For these subjects, the risk of anaphylaxis to latex during operations is high, and synthetic neoprene surgical gloves may be unacceptable to some surgeons (10). Moreover, vinyl plastic gloves may not be watertight and thus permeable to *herpes simplex* virus (4). Thus, it appears necessary to develop a glove manufacturing

process in which latex allergen is totally eliminated (14).

The purpose of our study was to obtain, by a revised manufacturing process, 1) gloves without allergenicity, and 2) a simple physical technique for estimating allergenicity.

MATERIAL AND METHODS

Five samples of gloves were made by Tulip Industries SA (Zone Industrielle, 54920 Villers la Montagne, France). The production line (11) was slightly modified to produce gloves A which were not washed, gloves B, C and D which were washed with demineralised water before drying (B at 22°C for 4 min; C at 22°C for 8 min, and D at 45°C for 4 min), and gloves E which were washed at 22°C for 4 min after drying. After manufacture the gloves were all washed under the same conditions with running demineralised water for 3 min to remove the corn powder, which contains proteins.

One glove of each sample was sterilized in the hospital at 120°C for 1 h, in saturated steam

(gloves A2, B2, C2, D2 and E2). They were examined by the manufacturer with the usual procedures: breaking load, stretch-breaking and electrical conductivity (or porosity). This last method serves to class surgical gloves according to their ability to prevent passage of electrical current with time (variable shielding effect). It was performed with 24 V 50 Hz alternating current. Aqueous solution containing 3.75 % NaCl raised to 37°C was used. The microammeter used was the Multimeter PM 2525 (Philips, Industrial and Electro-acoustic systems). The measurement was made when the stability level (plateau) of the curve was obtained.

To examine biochemical characteristics each glove was cut into pieces of about 3 to 5 cm² and then incubated 2 h with saline (1g glove/10 ml saline) at room temperature (20°C) with gentle stirring. The supernatant was then dispensed into aliquots and stored at -80°C until used. Protein concentration was measured by the Bio-Rad Protein assay. The lower limit of reliability was 1.25 µg/ml. Allergenic potency was

Table 1
Characteristics of gloves (A to E not sterilized; A2 to E2 sterilized)

	Protein content (µg/ml)	Allergenic potency (RAST-inhibition)	Electrical conductivity (mA)	Breaking load (MPa)	Breaking lengthening (%)
A	7.65	3.9	10.075 (63 min)*	27.81	774
A2	3	0.06	9.322 (57 min)	19.83	859
B	< 1.25	0.7	0.195 (11 min)	30.3	766
B2	UD	0.1	0.17 (9 min)	22.89	835
C	UD	0.25	0.113 (5 min)	28.17	788
C2	UD	UD	0.117 (5 min)	19.9	883
D	< 1.25	0.5	0.112 (7 min)	28.01	751
D2	UD	UD	0.088 (6 min)	20	832
E	3.2	2.2	0.665 (42 min)	27.9	803
E2	UD	0.17	0.547 (31 min)	20.91	877

UD: undetectable

* Time before reaching plateau level.

Table 2
Results of skin tests with A to E supernatants in Group I

Patients (sex-age)	RAST (class)	Positive control (codeine)	Commercial latex (1:100)	Prick tests (weal and flare in mm)				
				A	B	C	D	E
				(1:100)	(1:10)	(1:100)	(1:10)	(1:100)
				(1:10)	(1:10)	(1:10)	(1:10)	(1:10)
1 (F-34)	3	10-25	10-25	7-25	ND	0-0	5-16	0-0
2 (F-32)	2	15-40	7-35	6-35	ND	0-0	0-0	0-0
3 (F-39)	3	8-35	8-40	10-40	ND	10-35	ND	5-25
4 (F-34)	2	9-40	6-30	5-25	ND	0-0	5-20	7-35
5 (F-54)	2	12-45	7-30	7-35	ND	0-0	5-25	5-15
6 (F-36)	4	7-25	7-30	5-25	ND	0-0	5-20	5-20
7 (M-35)	3	7-20	5-20	5-18	ND	0-0	0-0	0-0
Mean $\pm$ SEM	Weal	9.7 $\pm$ 1.11	7.14 $\pm$ 0.59	6.43 $\pm$ 0.69	2.29 $\pm$ 1.54	1.43 $\pm$ 1.43	2 $\pm$ 1.36	3.14 $\pm$ 1.14
at 1:100	Flare	32.86 $\pm$ 3.6	30 $\pm$ 2.44	29 $\pm$ 2.94	8.57 $\pm$ 6.43	5 $\pm$ 5	7.86 $\pm$ 5.33	13.57 $\pm$ 5.31

ND: not done.

measured by RAST inhibition (9). This procedure compared the allergenic content of the glove supernatant with that of a reference extract of *Hevea brasiliensis* (commercial latex), used routinely in France for skin prick tests (Stallergenes SA, 94264 Fresnes Cedex, France). This extract was freshly reconstituted at 1:100 (w/v) concentration, which is the same as that used for skin tests. All assays were done at the same time, "blind", in coded tubes, by Stallergenes SA.

Skin prick tests with a Stallerpont® needle (Stallergenes SA) were performed on the anterior surface of the forearm in allergic patients after their informed consent. All had anaphylaxis to surgical gloves during operation, a positive skin test to commercial latex extract, and a positive RAST (class $\geq$ II) (Pharmacia France). The results are shown in Tables 2-4.

A negative control test was made with the diluent, a non-specific positive test with a 9% solution of codeine phosphate and a specific positive test with commercial latex extract at a concentration of 1:100 w/v. (see above).

Three groups of patients were tested (2 to 3 times for some patients):

- Group 1 with glove supernatants A to E.
- Group 2 with supernatants A2 to E2. The supernatants were diluted at 1:100 and 1:10 w/v concentrations after filtration through a 0.22 μ m Milipore® membrane. If the test at 1:100 w/v concentration was positive, with weal and flare diameter $\geq$ 2 mm than that of the negative control, then the test with 1:10 w/v concentration was not done.
- Group 3 through pieces of glove, which were distributed randomly and coded so that the operator made the test "blind".

Statistical analysis

The non-parametric Wilcoxon signed rank test and the Spearman correlation test were used to compare the weal diameters that were obtained with the positive control (codeine), the commercial latex extract, the 1:100 w/v concentration of supernatants and the prick tests through glove pieces. The electrical conductivity values were

Table 3
Results of skin tests with A2 to E2 supernatants in Group II

Patients (sex-age)	RAST (class)	Positive control (codeine)	Commercial latex (1:100)	Prick tests (weal and flare in mm)									
				A2		B2		C2		D2		E2	
				(1:100)	(1:10)	(1:100)	(1:10)	(1:100)	(1:10)	(1:100)	(1:10)	(1:100)	(1:10)
1 (F-37)	2	10-30	8-40	5-28	ND	5-15	0-0	0-0	5-12	6-20	ND	6-15	ND
2 (F-34)	3	10-25	10-25	7-15	ND	0-0	0-0	0-0	0-0	0-0	0-0	0-0	6-12
3 (F-33)	2	7-20	7-35	5-20	ND	0-0	0-0	0-0	0-0	0-0	0-0	0-0	4-12
4 (F-40)	3	11-35	11-40	0-0	7-20	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0
5 (F-46)	2	10-30	8-20	6-15	ND	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0
6 (F-36)	4	7-25	7-30	6-20	ND	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0
7 (F-54)	2	10-40	10-45	5-25	ND	0-0	6-30	0-0	0-0	0-0	0-0	5-15	ND
8 (M-35)	3	7-20	5-20	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0
Mean ± SEM	Weal	9 ± 0.6	8.25 ± 0.7	4.25 ± 0.96	0.62 ± 0.62	0 ± 0	0.75 ± 0.75	1.38 ± 0.91					
at 1:100	Flare	28.12 ± 2.49	31.88 ± 3.4	15.38 ± 3.7	1.88 ± 1.88	0 ± 0	2.5 ± 2.5	3.75 ± 2.45					

ND: not done.

transformed to natural logarithms to obtain a normal figure distribution.

RESULTS

Protein content and allergenic potency

Table 1 shows that the protein content and allergenic potency were clearly lower in washed gloves (B, C, D) and lower still when washing time was doubled (B vs C). Increased water temperature had no significant effect (B vs D).

There was a rough correlation between protein content, allergenic potency and electrical conductivity. However, the RAST inhibition results showed that some allergenic material remains even though the protein content may not be detectable (B2, C and E2).

Skin tests

All results were confirmed by skin tests, (Table 2) and showed that there was no significant difference between commercial latex extract and unwashed glove supernatant (A), which gave clear positive skin tests at 1:100 w/v concentration. However, tests with commercial latex extract and with supernatant A (at 1:100 w/v concentration) were more reactive than any other supernatant ($P \leq 0.02$ to $P < 0.009$).

All seven patients in Group 1 were positive with the supernatant A at 1:100 w/v against one of seven with C (chi-square with Yates' correction: 7.3, $P < 0.01$).

Effect of sterilization

The effect of glove sterilization at 120°C was obvious as can be seen in Table 1. The protein content was undetectable in four of five gloves (B2, C2, D2, and E2) and the allergenic potency decreased at least 7-fold. However, sterilization was not sufficient to suppress allergenicity of unwashed gloves (A2) as the weal diameter of A2 (Table 3) was higher than B2 ($P = 0.022$), C2 ($P = 0.014$), D2 ($P = 0.023$) and E2 ($P = 0.04$).

Table 4
Results of skin tests from gloves in Group 3

Patients (sex-age)	RAST (class)	Positive control (codeine)	Commercial latex (1:100)	A	A2	B	B2	C	C2	D	D2	E	E2
1 (F-46)	2	8-40	8-25	5-20	0-0	5-15	0-0	0-0	0-0	0-0	0-0	5-20	4-15
2 (F-34)	3	10-25	10-25	6-12	0-0	0-0	0-0	3-16	0-0	0-0	0-0	6-15	0-0
3 (F-36)	4	7-25	7-30	0-0	4-12	0-0	0-0	0-0	0-0	0-0	0-0	7-25	0-0
4 (F-54)	2	10-40	10-45	7-30	0-0	4-15	0-0	2-15	3-15	4-20	0-0	6-25	3-25
5 (M-35)	3	8-20	8-20	0-0	0-0	0-0	0-0	4-13	0-0	5-15	0-0	0-0	0-0
6 (F-35)	1	7-30	7-30	6-15	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0-0
7 (F-48)	3	7-20	7-30	5-15	5-12	4-15	0-0	5-20	0-0	5-12	0-0	6-20	4-11
Mean±SEM	Weal	8.14±0.51	8.14±0.51	4.14±1.1	1.29±0.84	1.86±0.88	0±0	2±0.79	0.43±0.43	2±0.95	0±0	4.29±1.13	1.57±0.75
at 1:100	Flare	28.57±3.22	29.29±2.97	13.14±4.04	3.43±2.21	6.43±3.03	0±0	9.14±3.33	2.14±2.14	6.71±3.29	0±0	15±4.08	7.29±3.78

The number of patients in each group was small and it was not possible to reach a level of significance by comparing the weals from supernatants A to E with those from A2 to E2. We therefore verified that Groups 1 and 2 were comparable for the values obtained with the positive codeine control and the commercial latex extract. The means ($\pm$ SEM) were 9.7 ± 1.1 vs 9 ± 0.6 mm and 7.14 ± 0.59 vs 8.25 ± 0.7 mm, respectively. There was no statistical difference, either by Student *t* test or Wilcoxon test.

Thus, it was possible to compare the 35 weals of A to E at 1:100 w/v concentration with the 40 weals of A2 to E2 at 1:100 w/v concentration. The mean of A to E was 3.06 ± 0.61 mm and for A2 to E2 was 0.68 ± 0.29 mm. This difference was significant, with $P < 0.002$ by Wilcoxon test.

In Group 3 (Table 4), the differences between weals can be seen to be either significant (A vs A2, $P < 0.04$ and E vs E2, $P = 0.03$) or approaching significance (B vs B2: $P = 0.054$; C vs C2: $P = 0.07$ and D vs D2: $P = 0.054$).

When the weal values were recombined the means became 2.86 ± 0.45 mm for A to E and 0.66 ± 0.25 mm for A2 to E2. The difference was significant, ($P < 0.0001$ with the Wilcoxon test).

In Groups 1 and 2 the Spearman test showed a significant correlation of $R = 0.68$, $n = 10$, $P = 0.029$ between the means of the weal size and electrical conductivity and between the electrical conductivity and percentage of positive patients (at 1:100 or 1:10), with $R = 0.73$, $n = 10$, $P = 0.017$. The best graph was obtained when the electrical conductivity values were transformed into natural logarithms (Fig. 1).

DISCUSSION

Anaphylaxis caused by the use of latex surgical gloves is now a well-known side effect. A major problem is how to operate patients who are allergic to latex; plastic gloves do not have the same good mechanical properties. It has also been shown that about 1% of vinyl gloves were permeable to *Herpes* virus, though this was not so with those made from latex (4).

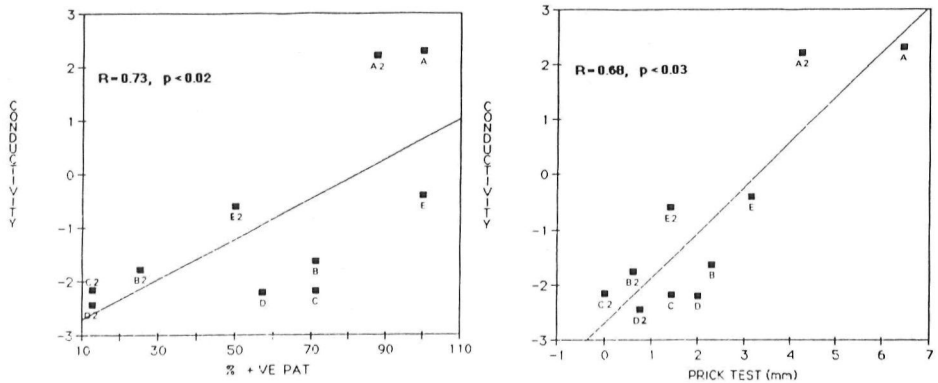


Fig. 1. Correlation between electrical conductivity of gloves (expressed as Ln mA) in Groups 1 and 2 and (left) the percentage of skin-test positive patients and (right) mean size of weals.

There have been many reports that several proteins (2 to 100 kD) were the allergenic material of latex gloves (7, 14, 16), thus it is not surprising that Morales et al. (4) were able to suppress the allergenic water-soluble material by washing. However, the allergenicity of latex gloves may differ from manufacturer to manufacturer (14).

The purpose of this study was to modify the manufacturing method to produce surgical gloves without allergenicity. When the washing period during production was increased the protein content, allergenic material (RAST inhibition) and frequency of positive skin tests decreased, though some patients still showed positive skin tests.

We tried glove sterilization by the usual steam method, allergy to gloves being exceptional in some countries where surgical gloves are re-used 3 to 6 times after sterilization (Dr Ratti, Paraguay, personal communication). It was clear that protein content and allergenic material were decreased by this supplementary step (C2 and D2 gloves, Table 1) and skin tests with these glove supernatants were negative (D2) or nearly positive (≥ 2 mm than the negative control) at the 1:10 concentration (C2 Table 3). A prospective study is now in progress with gloves C2 and D2 in allergic patients who must undergo operation.

As manufacturers need a quality control procedure for glove allergenicity that does not re-

quire human serum or skin tests, we measured some of the physico-chemical properties of gloves. Protein content proved to be inadequate, since skin tests were positive even if the protein content was undetectable. Turjanmaa et al. (14) have previously shown that the amount of protein eluting from gloves does not predict the allergenicity of the particular product.

Electrical conductivity may be another simple control procedure, as it is highly dependent on such water-soluble substances as proteins and salts like sulphonates, sulphates, polyphosphates, etc., added during the manufacturing process (11). As some substances are water-absorbent, the electrical conductivity may increase with time, reaching the plateau level used in our work.

Although there was a significant correlation between electrical conductivity and skin tests, there was some overlap as B and B2 had approximate the same conductivity (0.195 vs 0.17 mA) but the mean weal size of B was 3.7 times higher than that of B2.

More studies on gloves with conductivity lower than 0.1 mA would be necessary before this parameter could be accepted as useful.

In conclusion, it appears that two steps are necessary to decrease or suppress the allergenicity of the gloves: 1) to wash the gloves after mould-forming, before they are dried and 2) sterilization with steam. Both steps are practicable in the manufacturing process.

ACKNOWLEDGEMENT

We thank Mrs C. Roy and Mrs C. Chambeyron for their technical help.

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